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Short Communication

Broad-few-leaflets and outwardly curved wings: two new mutants of chickpea

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With 1 figure and 1 table

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Abstract

This study was aimed at the induction of morphological mutations for increasing genetic variability and making available additional genetic markers for linkage studies in chickpea (*Cicer arietinum* L.). A wilt-resistant, well-adapted chickpea cultivar of central India, 'JG 315' (Jawahar gram 315), was used for the induction of mutations. Seeds presoaked in distilled water for 2 h were treated with ethyl methane sulphonate (EMS) using six different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%) and two different durations (6 and 8 h). Several morphological mutants were identified in M_2. One of the mutants, isolated from a treatment of 0.3% EMS for 8 h, had five to nine large leaflets per leaf in comparison with 11–17 normal-sized leaflets per leaf observed in the parental cultivar 'JG 315'. The mutant was designated broad-few-leaflets. Many leaves of this mutant showed a cluster of three to five overlapping leaflets at the terminal end. The other mutant, designated outwardly curved wings, was isolated from the 0.5% EMS treatment for 6 h. In this mutant, the wings were curved outwards, exposing the keel petal, while the wings in typical chickpea flowers are incurved and enclose the keel. The lines developed from the broad-few-leaflets and outwardly curved wings mutants were named JGM 4 (Jawahar gram mutant 4) and JGM 5, respectively. Inheritance studies indicated that each of these mutant traits is governed by a single recessive gene. The gene for broad-few-leaflets was designated *bfl* and the gene for outwardly curved wings was designated *ocw*. The locus *bfl* was found to be linked with the locus *lg* (light green foliage) with a map distance of 18.7 ± 6.3 cM.

Key words: *Cicer arietinum* — ethyl methane sulphonate — inheritance — linkage — morphological mutants

The cultivated chickpea (*Cicer arietinum* L.) is considered to have a narrow genetic base, as little polymorphism has been detected for isozyme, RFLP and RAPD markers (Gaur and Slinkard 1990, Simon and Muehlbauer 1997). Though the use of these markers in linkage studies is possible, by using interspecific mapping populations, limited success has been achieved in the development of a detailed genome map of chickpea (Gaur and Slinkard 1990, Kazan et al. 1993, Simon and Muehlbauer 1997). The discovery of a high level of polymorphism in microsatellites or simple sequence repeats has renewed interest in the genome mapping of chickpea in recent years and considerable progress has been made in mapping markers based on these hyperpolymorphic regions (Winter et al. 2000, Cho et al. 2002).

A high level of variability is seen in the cultivated chickpea for morphological traits, but this could be a reflection of the expression of a limited number of mutant genes. A single mutant gene may cause marked changes in the appearance of the plant, e.g. *slv* for simple leaf (Ekbote 1937), *ml* or *mlv* for multipinnate leaf (Pundir et al. 1990) and *fas* for stem fasciation (Gaur and Gour 1999).

The spontaneous and induced mutants accumulated in chickpea over the years constitute a valuable genetic resource for its improvement. Some of the morphological mutants, such as double-podding (Kumar et al. 2000) and compact growth habit (Lather 2000), have already been found to be promising in breeding. Any addition to the pool of genetic markers will be welcomed, because few genes for morphological traits have so far been assigned to linkage groups in chickpea (Gaur and Slinkard 1990, Kazan et al. 1993, Simon and Muehlbauer 1997, Cho et al. 2002). This study was aimed at inducing mutants in chickpea that are particularly suitable for use in linkage studies. This report describes two new mutants that have been added to the genetic stock of chickpea.

A wilt-resistant cultivar of chickpea, 'JG 315', widely grown in central India, was selected for mutagenesis. Seeds were soaked in distilled water for 2 h and then treated with six different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%) of ethyl methane sulphonate (EMS) for two separate durations (6 and 8 h). The M_1 from each treatment was harvested separately. The M_2 was carefully examined at all growth stages for identification of mutants.

Several morphological mutants were isolated from the M_2, three of which have already been described (Gaur and Gour 1999). Two additional mutants, one for leaf type and the other for flower morphology, are described here.

The chickpea leaf is typically pinnate with, on an average, 11–17 leaflets with small pedicels attached to the rachis. There is usually one leaflet on the terminal end of the rachis. However, mutants with simple leaf (Ekbote 1937) and multipinnate leaf (Pundir et al. 1990) have also been reported. The leaf-type mutant obtained in this study, designated broad-few-leaflets, had five to nine leaflets per leaf compared with 11–17 leaflets per leaf in the parental cultivar 'JG 315'. The leaflets were comparatively larger in size (Fig. 1a). A cluster of three to five overlapping leaflets was observed at the terminal end in some leaves. The line developed from this mutant was named JGM 4 (Jawahar gram mutant 4).

The chickpea flower is typically papilionaceous, in which the standard petal is broad and clawed, and the wings are incurved and enclose the keel. The flower mutant identified in this study

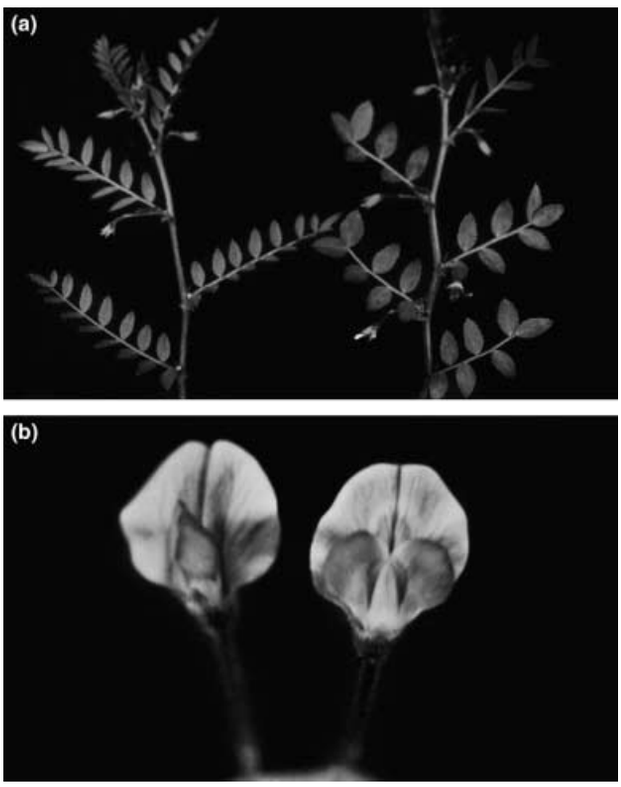

Fig. 1: (a) Branch of the chickpea cultivar 'JG 315' with normal leaflets (left) and that of an induced mutant of 'JG 315' with *broad-few-leaflets* (right). (b) Typical chickpea flower with incurved wings enclosing the keel (left) and a flower of an induced mutant of 'JG 315' with *outwardly curved wings*, exposing the keel (right)

had outwardly curved wings exposing the keel (Fig. 1b). This trait was fully expressed in old flowers. The mutant line was named JGM 5.

The line JGM 4 was crossed with JGM 5 and eight other chickpea marker lines for the study of inheritance and linkage relations with other traits. The marker lines included ICC 4929 (pink-striped white flower, double-podding), ICC 4957 (green seed coat and cotyledons), ICC 5325 (light-green foliage), ICC 5434 (prostrate growth habit), ICC 5783 (purple foliage), ICC 8540 (black seed coat), ICC 12450 (blue flower, pea seed shape) and ICC 15566 (glabrous shoot, leaf necrosis). A 3 : 1 goodness-of-fit ratio for plants with normal leaflets and plants with broad-few-leaflets was observed in seven of the nine families (Table 1). The distorted segregation observed in two of the crosses could be attributed to chance. Thus, the results indicate that a single recessive gene, designated *bfl*, is responsible for the broad-few-leaflet mutant.

A linkage was detected between the loci *bfl* and *lg* (light-green foliage) from the cross JGM 4 (broad-few-leaflets with normal green foliage) × ICC 5325 (normal leaflets with light-green foliage). Of 234 plants examined in the F_2, 120 had normal leaflets with normal green foliage, 66 had normal leaflets with light-green foliage, 46 had broad-few-leaflets with normal green foliage and two plants had broad-few-leaflets with light-green foliage. The data were analyzed using the computer program LINKAGE1 (Suiter et al. 1983). Each locus gave a 3 : 1 goodness-of-fit Mendelian ratio. A contingency χ^2-value of 18.15 was estimated, which was highly significant ($P = 0.00002$) and indicated that *bfl* and *lg* are linked. A recombination value of 18.7 ± 6.3 cM was estimated between the two loci.

The inheritance of outwardly curved wings was studied in the F_2 of five crosses. These included crosses of JGM 5 with JGM 4 and four marker lines, namely ICC 4957, ICC 5316 (multipinnate leaf, pea seed shape), ICC 5325 and ICC 5783. The incurved wings were dominant outwardly curved wings and showed monogenic segregation (Table 1). The recessive gene responsible for outwardly curved wings was designated *ocw*.

Table 1: Goodness-of-fit χ^2-tests for 3 : 1 single-locus F_2 segregation of the mutant traits broad-few-leaflets and outwardly curved wings in chickpea

Cross	F_2 phenotype		Goodness-of-fit		Heterogeneity	
			χ^2	P	χ^2	P
Normal leaflets vs. *broad-few-leaflets*						
	Normal leaflets	*Broad-few-leaflets*				
JGM 4[1] × ICC 4929	152	40	1.78	0.18		
JGM 4 × ICC 4957	70	20	0.37	0.54		
JGM 4 × ICC 5325	186	48	2.51	0.11		
JGM 4 × ICC 5434	120	58	5.46	0.02		
JGM 4 × ICC 5783	200	66	0.01	0.74		
JGM 4 × ICC 8540	188	42	5.57	0.02		
JGM 4 × ICC 12450	138	42	0.27	0.60		
JGM 4 × ICC 15566	155	55	0.16	0.69		
JGM 4 × JGM 5	172	50	0.73	0.39		
Pooled data	1381	421	2.58	0.11	14.27	0.07
Incurved wings vs. *outwardly curved wings*						
	Incurved wings	*Outwardly curved wings*				
JGM 5[2] × ICC 4957	144	31	4.95	0.03		
JGM 5 × ICC 5316	130	38	0.51	0.48		
JGM 5 × ICC 5325	215	72	0.00	1.00		
JGM 5 × ICC 5783	152	38	2.53	0.47		
JGM 4 × JGM 5	160	62	1.02	0.31		
Pooled data	801	241	1.95	0.16	7.07	0.13

[1] JGM 4 = an induced mutant of JG 315 with broad-few leaflets.
[2] JGM 5 = an induced mutant of JG 315 with outwardly curved wings.

Though inheritance has been reported for a large number of morphological traits (reviewed by Muehlbauer and Singh 1987), only a few have been assigned to the linkage groups of chickpea. These include three loci, *slv* (simple leaf), *ml* or *mlv* (multipinnate leaf) and *gr* (green seed coat and cotyledon), mapped by Gaur and Slinkard (1990); five loci, *hg* (prostrate growth habit), *gst* (epicotyl colour), *P (*flower colour), *T3 (*seed coat colour) and *Ps* (seed surface), mapped by Kazan et al. (1993); and four loci, *sfl* (double podding), seed size, pigmentation, seed type and crude fibre, mapped by Cho et al. (2002). There is an obvious need to assign additional morphological trait loci to the rapidly developing molecular map of chickpea.

The mutants reported here would be very useful in mapping studies, as they are easy to score. The linkage identified between *bfl* and *lg* is a welcome addition to our knowledge of genetic linkages in chickpea.

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